Poster Reprint

ASMS 2022
Poster number WP378

Accurate Mass Library for Natural Products Based on Compounds Identified in Hemp CBD Oil

Sofia Nieto; Matthew Curtis; Paul Contreras and Andrew McEachran

Agilent Technologies, Santa Clara, CA

Introduction

The use of hemp and CBD oils has become increasingly popular in many parts of the world. One of the important directions of the chemical analysis of hemp and hemp products includes an exploration of the chemical composition of different hemp strains to identify compounds with bioactive properties. Concentrated hemp CBD oils are very complex samples, therefore, a comprehensive GCxGC approach may be beneficial to ensure the chromatographic separation of individual components, while high resolution accurate mass GC/MS helps to reduce the ambiguity in the compound identification. Here we describe the development of the accurate mass EI library for natural products that can be used with 1D chromatographic separation. This study also provides examples of the target and non-target screening workflows using this library.

Figure 1. Agilent 7250 GC/Q-TOF

Experimental

Five different hemp CBD oil samples were analyzed using a high-resolution GC/Q-TOF in either 1D or 2D comprehensive GCxGC configuration using the ZOEX ZX2 thermal modulator. A 5% phenyl, 30m column was used for the 1D data while the GCxGC configuration was a 5% phenyl 30m column coupled to a 2.8m DB-HeavyWAX. The data were acquired at 70eV. More detailed instrumental parameters are shown in Table 1.

GC and MS Conditions	2D	1D
MS	7250 Q-TOF	
GC	7890	
Inlet	MMI, 4-mm UI liner single taper with wool	
Inlet temperature	280°C	
Injection volume	1 µL	
Columns	Primary: DB-5MS UI, 30 m x 0.25 mm x 0.25 µm	DB-5MS UI, 30 m x 0.25 mm x 0.25 µm
	Secondary: DB-HeavyWax, 2.8 m x 100 µm x 0.1 µm	-
Oven temperature program	60°C for 5 min; 3°C/min to 290°C, 25 min hold	60°C for 5 min; 4°C/min to 300°C, 7 min hold
Carrier gas	Helium	
Column flow	1 mL/min constant flow	
Modulation period	6 sec	-
Cold jet flow	13 L/min	-
Hot jet temperature	300°C	-
Hot jet duration	320 ms	-
Transfer line temperature	280°C	
Quadrupole temperature	150°C	
Source temperature	200°C	
Electron energy	70 eV	
Emission current	5 µA	
Spectral acquisition rate	50 Hz	5 Hz
Mass range	40 to 650 m/z	

Table 1. Instrument and method parameters

The retention indices were calculated based on the alkane ladder to assist compound identification and library curation. The GC/Q-TOF data were processed using the Unknown Analysis and MassHunter Quantitative Analysis Software version 10.2, MassHunter Qualitative Analysis Software version 10. GC Image version 2.9r2 was used to visualize the 2D data.

Creating the Accurate Mass Library of Natural Products

The objective of the present study is to create a comprehensive accurate mass PCDL (Personal Compound Database and Library) based on hemp CBD oil samples for higher confidence fast screening in 1D GC configuration. In order to achieve adequate chromatographic separation of these complex samples, the data were collected using GCxGC configuration. The GCxGC data were visualized using GC Image software and compounds were tentatively identified using NIST17 and NIST20 libraries. On a 2D plot one can clearly see the separation of the different compound classes (Figure 2) and how the RT of a compound in a second dimension can provide an additional confidence in compound identification.

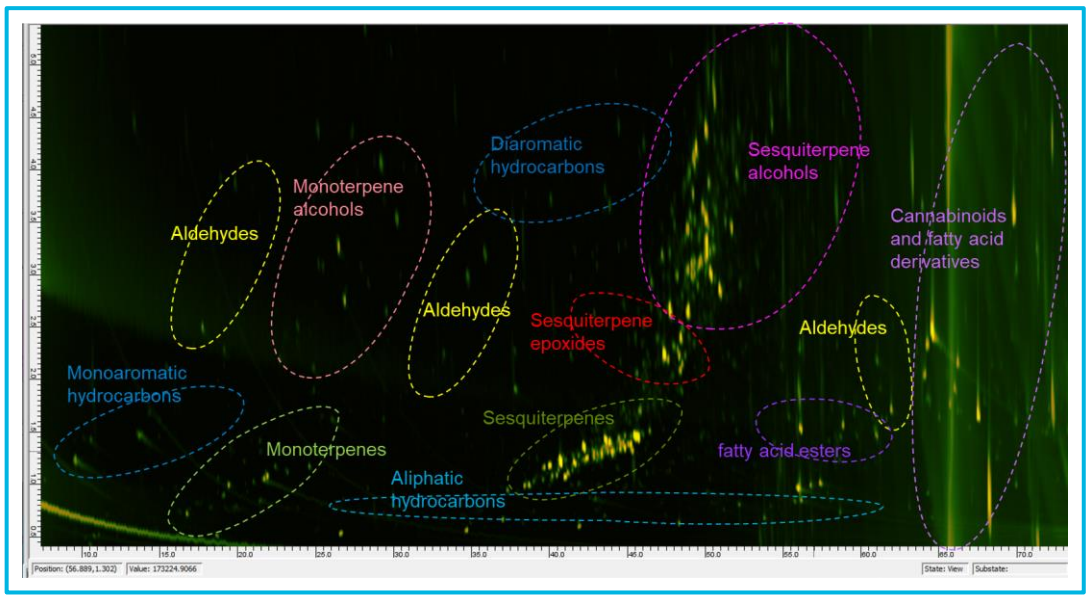

Figure 2. Compound classes mapped on GCxGC/Q-TOF chromatogram of a CDB oil sample

Moreover, accurate mass, isotope ratios, as well as retention indices (RI) were also used to further increase confidence in identifying the components of a hemp CDB oil sample. The fragment formula annotation of the compound spectra was performed using MassHunter Qualitative Analysis Software (Figure 3A). The annotated spectra were exported to the PCDL after curation and automatic conversion of the measured m/z values to the theoretical values based on the elemental compositions of the individual ions (Figure 3B). Whenever a precise identification of an isomer was not possible, a compound would be assigned an indexed molecular formula instead of a name. The current PCDL contains approximately 350 compounds.

Distribution of the different compound classes in the PCDL including, whenever possible, those identified down to the formula, is shown in Figure 4. Monoterpenoids, sesquiterpenoids and variety of alcohols represented almost a half of the total number of spectra included in the PCDL.

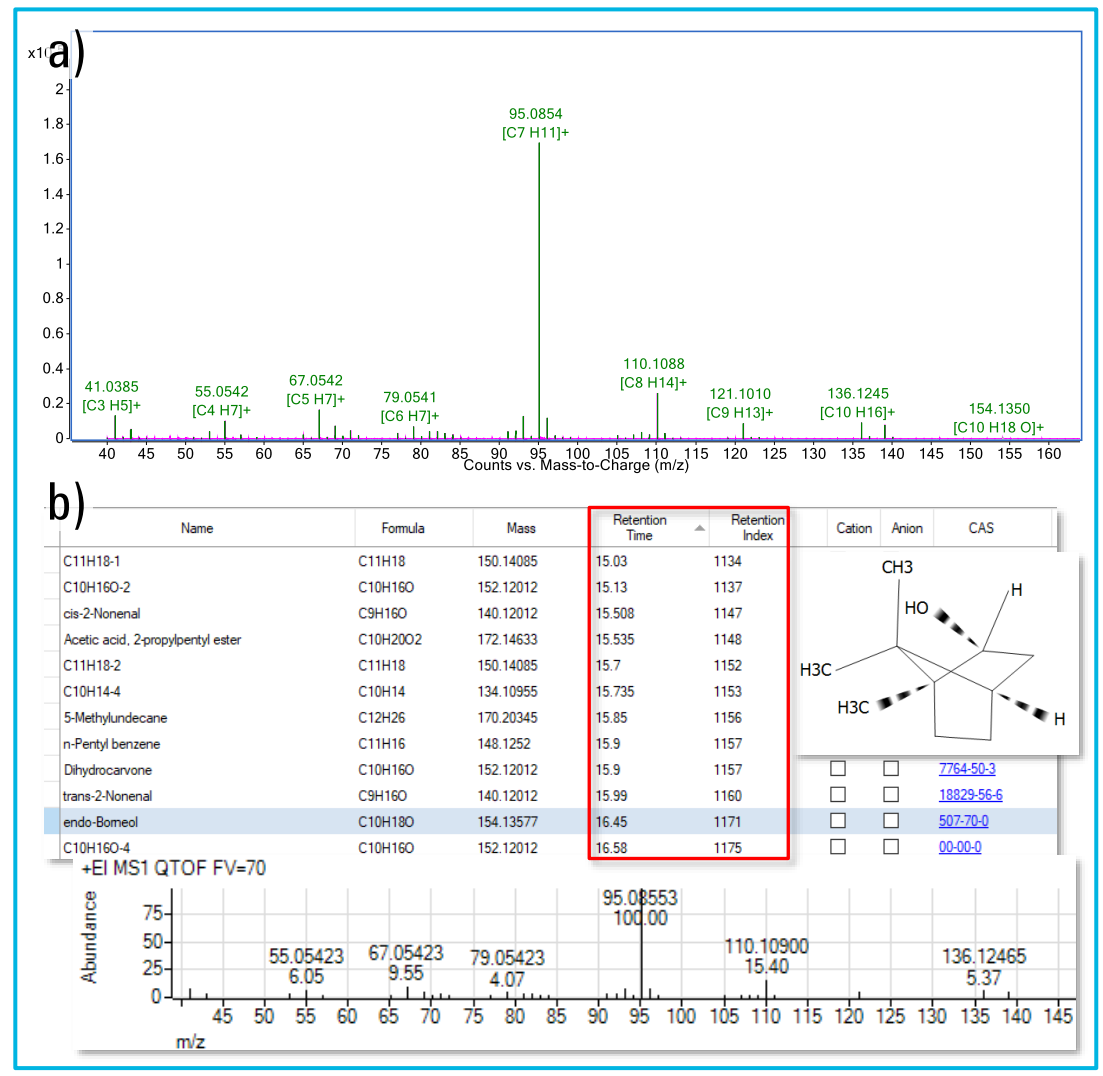

Figure 3. (a) Fragment formula annotation of spectrum is an important step in creating a high quality accurate mass library. (b) The PCDL of Hemp and Natural Products includes both Retention Times and Retention Indices. All the spectra have theoretical m/z of the fragment ions.

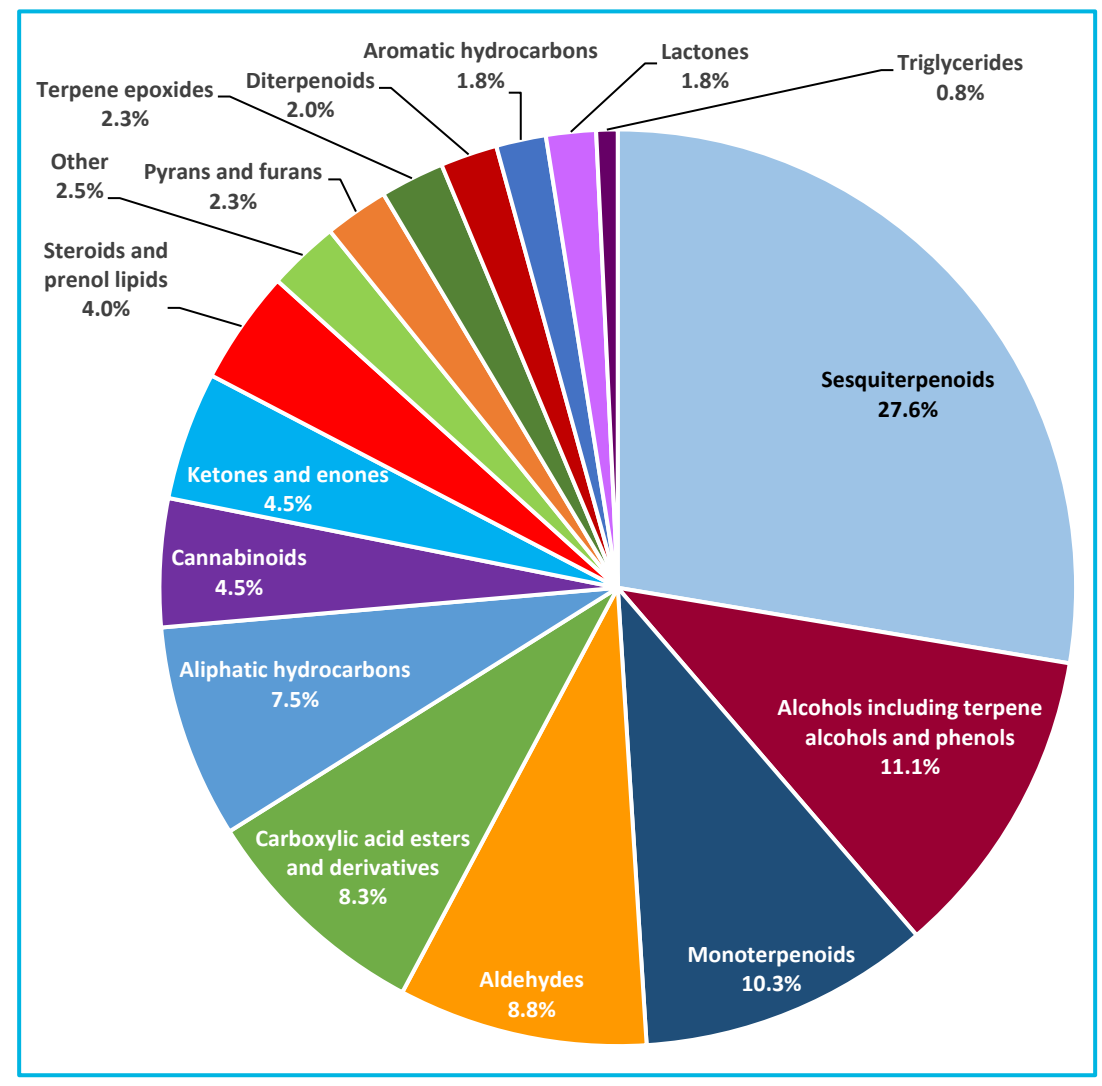

Figure 4. Compound classes in the PCDL

Target and Non-target Workflows Using the Accurate Mass Library

In order to test both target and non-target workflows with the Natural Products PCDL, cannabis extracts as well as hemp CBD oils data were acquired in 1D GC. The screening conditions have been optimized for each workflow separately to ensure the minimum detection of false positives and false negatives. The examples of non-target and target approaches are shown in Figures 5 and 6, respectively.

In both cases the majority of the true hits has been detected with a high library match score of >80 (Tables 2 and 3). When using the non-target approach, the number of the true hits with the library match score <80 was significantly higher as compared to the target screening. On the other hand, when using target screening, the vast majority of the true hits had the library match score >90. The library match score is one of the key parameters in the screening method, therefore, it might be helpful to keep in mind this difference between the two approaches.

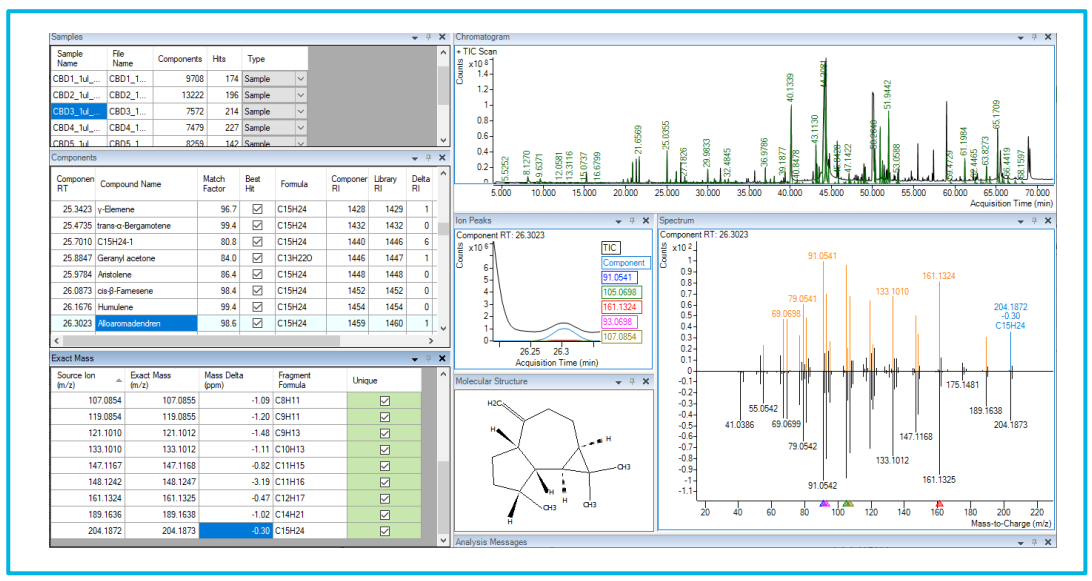

Figure 5. Non-target screening in Unknowns Analysis. Exact Mass feature helps to eliminate the false positives by examining if the accurate mass ions in a spectrum fit the subset of the molecular formula of the hit.

Match Score	CBD1	CBD2	CBD3	CBD4	CBD5	CBD6	Cannabis Extract
>90	45.4	48.5	42.5	39.2	41.5	49.3	45.2
80-90	20.1	20.4	24.8	24.2	24.6	23.3	18.3
<80	34.5	31.1	32.7	36.6	33.8	27.4	36.5

Table 2. Percentage of confirmed compounds observed via non-target screening by library match score threshold across CBD oil and cannabis samples

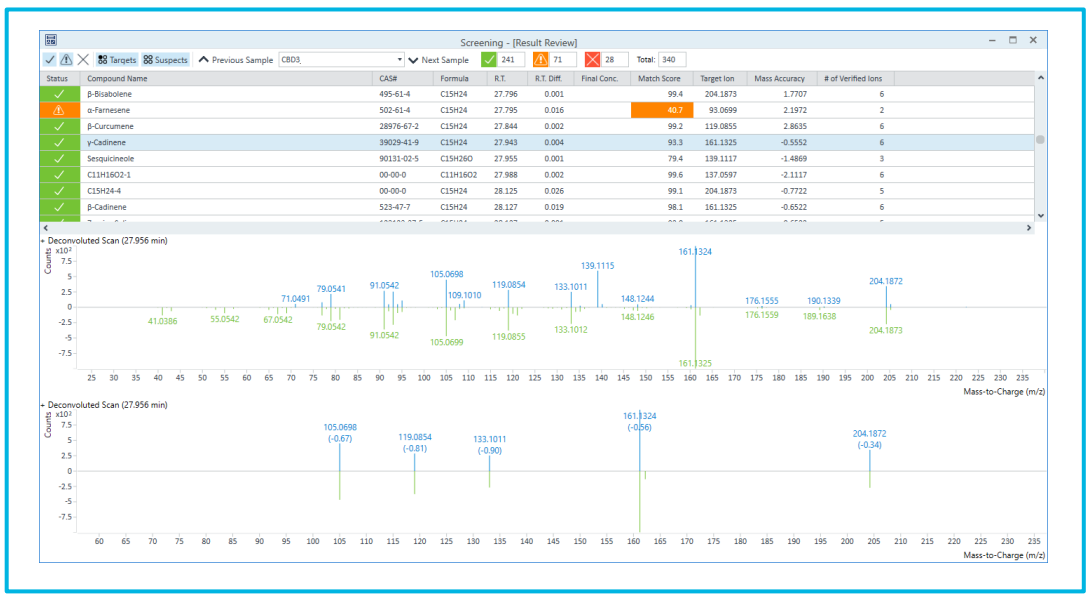

Figure 6. Target screening summary window in MassHunter Quantitative Analysis

Match Score	CBD1	CBD2	CBD3	CBD4	CBD5	CBD6	Cannabis Extract
>90	69.0	63.8	71.4	76.3	64.4	63.1	50.4
80-90	20.5	19.1	16.9	12.1	16.7	20.9	23.9
<80	10.5	17.0	11.7	11.6	19.0	16.0	25.6

Table 3. Percentage of confirmed compounds observed via target screening by library match score threshold across CBD oil and cannabis samples

The target and non-target screening approaches using the accurate mass Natural Products PCDL yielded a similar number of the identified compounds (Table 4), although in all samples target screening identified a slightly higher number of the true hits.

Workflow/ Sample Name	CBD1	CBD2	CBD3	CBD4	CBD5	CBD6	Cannabis Extract
Target screening	187	201	230	233	169	172	112
Non-target screening	174	196	214	227	142	146	104

Table 4. Number of the true hits identified in target vs non-target screening approaches

Conclusions

- A new accurate mass library for cannabis and other natural products has been created using a hemp CBD oil sample analyzed using the GCxGC/Q-TOF
- Both target and non-target workflows are compatible with the Natural Products PCDL with the target screening approach being slightly more sensitive

https://explore.agilent.com/asms

This information is subject to change without notice.

DE03161722

Published in USA, May 25, 2022

Agilent

Trusted Answers